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(54) DIOXINS ANALYZING METHOD

(57)Abstract:
PROBLEM TO BE SOLVED: To analyze dioxins accumulated in the bodies of animals by detecting a specimen more easily sampled than blood and analyzed with the approximately same accuracy as a blood specimen and using dioxins contained in the specimen as an index.
SOLUTION: Saliva is collected from an animal as the specimen. With dioxins contained in the saliva as the index, dioxins accumulated in the body of the animal are analyzed.

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CLAIMS

[Claim(s)]
[Claim 1] Analytical method of the dioxin accumulated in the inside of the body of an animal characterized by analyzing the dioxin accumulated in the inside of the body of said animal by making into an index the dioxin contained in the saliva extracted from the animal.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the analytical method of the dioxin accumulated in the inside of the body of an animal (especially Homo sapiens).
[0002]
[Description of the Prior Art] Generally, Pori chlorination dibenzo-Para-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) are collectively called "dioxin", and the matter in which the same toxicity as dioxin is shown like coplanar PCB (coplanar PCB) is called the "dioxin analogue." Moreover, PCDD, PCDF, and coplanar one PCB may be collectively called "dioxin." Below, suppose that it calls PCDD, PCDF, and coplanar one PCB "dioxin" collectively.
[0003] It is checked that PCDD and PCDF have various toxicity, such as acute toxicity, chronic toxicity, carcinogenic, reproduction toxicity, teratogenicity, and immunotoxicity, from the result of an animal experiment. Moreover, it is known that coplanar one PCB will cause a loss weight, thymus gland withering, reproduction toxicity, porphin **, etc. Thus, in order for dioxin to show various toxicity to an animal including Homo sapiens, it is important for it to analyze the are recording situation to the Homo sapiens inside of the body of dioxin, when maintaining human health.
[0004] 90% or more of the intake depends the dioxin which Homo sapiens takes in in everyday life on a meal, i.e., taking orally. Although dioxin hardly melts into water, since it has the property to be easy to melt into a fat, the dioxin by which the ingestion was carried out is mainly distributed over blood, liver, muscles, the skin, and fat tissue, and it is accumulated especially in liver and fat tissue. In any much dioxin shall be accumulated between liver and fat tissue changes with animal species, and much dioxin is accumulated by fat tissue in Homo sapiens. Moreover, the dioxin once accumulated in the inside of the body is hard to be metabolized, with the drug-metabolizing enzyme of liver microsome, a polar substance is metabolized slowly and many of metabolite is excreted in bile. Moreover, discharge to the outside of the body of dioxin is mainly performed by elimination of stools, and what is depended on urine is lessened.
[0005] Therefore, although it is desirable to analyze the are recording situation of the dioxin in liver or fat tissue in order to analyze the are recording situation to the Homo sapiens inside of the body of dioxin, the sampling of the specimen from liver or fat tissue is difficult. Then, generally, blood is sampled and the are recording situation to the Homo sapiens inside of the body of dioxin is analyzed by making concentration of the dioxin in blood into an index.
[0006]
[Problem(s) to be Solved by the Invention] However, since about 100ml is needed as blood volume when measuring the concentration of the dioxin in blood, it cannot be targeted at infants. Moreover, since it is that to which blood extraction is performed as part of a medical action, it is difficult to extract many specimens.
[0007] Then, this invention aims at offering the approach of analyzing the dioxin accumulated in the inside of the body of an animal by making into an index the dioxin which contains the specimen which can be analyzed in a precision comparable as the case where a sampling is easy and makes blood a specimen rather than blood as a specimen at the time of analyzing the dioxin

accumulated in the inside of the body of an animal in a header and its specimen.
[0008]
[Means for Solving the Problem] In order to solve the above-mentioned technical problem, analytical method of the dioxin accumulated in the inside of the body of the animal offered by this invention is characterized by analyzing the dioxin accumulated in the inside of the body of said animal by making into an index the dioxin contained in the saliva extracted from the animal.
[0009]
[Embodiment of the Invention] Hereafter, this invention is explained to a detail. In this invention, the dioxin analogue which shows the same toxicity as PCDD or PCDF like coplanar PCB (coplanar PCB) besides Pori chlorination dibenzo-Para-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) is contained in "dioxin."
[0010] PCDD is a compound which has a dibenzo-Para-dioxin frame, and is the compound with which one piece or plurality was permuted by the chlorine atom among the hydrogen atoms of the 1-4th place and the 6-9th place. Moreover, PCDF is a compound which has a dibenzofuran frame and is the compound with which one piece or plurality was permuted by the chlorine atom among the hydrogen atoms of the 1-4th place and the 6-9th place. Moreover, PCB is a compound which has a biphenyl frame, one piece or plurality is the compound permuted by the chlorine atom among the hydrogen atoms the 2-6th place and like 2'-6', and coplanar one PCB is in the condition (coplanarity: Coplanarity) that there are two phenyl groups in the same flat surface in three dimensions in PCB (a non orthochromatic object, a mono-orthochromatic object, and JIORUSO object).
[0011] In this invention, in case the dioxin accumulated in the inside of the body of an animal is analyzed, the saliva extracted from the animal concerned is used as a specimen. The saliva used as a specimen is saliva which the animal used as the candidate for analysis secretes, and is extracted from the animal used as the candidate for analysis. It is not limited especially as long as the animal used as the candidate for analysis can secrete saliva, and as the example, mammalians, such as Homo sapiens, an ape, a dog, a cat, a rat, a guinea pig, and a rabbit, are mentioned.
[0012] The saliva used as a specimen is extractable according to a conventional method from the inside of the oral cavity of the animal used as the candidate for analysis. In case saliva is extracted, it is desirable to clean beforehand the inside of the oral cavity of the animal used as the candidate for analysis by brushing etc. so that the dioxin of the food origin which the animal concerned took in may not mix into the saliva which the animal used as the candidate for analysis secretes. Any of the saliva secreted under the saliva secreted under the conditions of not stimulating, and owner stimulus conditions are sufficient as the saliva to extract. As for the instrument used for extraction of saliva, it is desirable to fully wash by an acetone, toluene, etc. for a residual-agricultural-chemicals trial so that the dioxin adhering to the instrument concerned may not mix into saliva. The output of saliva is a complement at analysis of the dioxin contained in saliva, and is usually 10ml - about 10ml of numbers.
[0013] In this invention, in case you analyze the dioxin accumulated in the inside of the body of an animal, let the dioxin contained in the saliva extracted from the animal concerned be an index. The qualitative-analysis result or quantitative-analysis result of dioxin specifically contained in the saliva extracted from the animal concerned can be made into an index.
[0014] The qualitative analysis and quantitative analysis of dioxin which are contained in saliva can be carried out by the selected-ion-detection method (the SIM method) which used for example, high-resolution mold gas chromatograph-mass spectrometer (HRGC/HRMS). Under the present circumstances, to the saliva sample, the internal standard (for example, several sorts of coplanar PCB which carried out the indicator by 13C or 37Cl(s) when coplanar one PCB was a candidate for analysis) corresponding to the dioxin it is incomparable for the candidate for analysis is added. Moreover, after adding an internal standard to a saliva sample, pretreatment is performed and the analysis interfering substance is removed from the saliva sample. After carrying out solvent extraction (for example, dichloromethane extract) for example, of the saliva sample and condensing an extract, pretreatment is ****(ed) to a hexane and, subsequently can be performed by carrying out multilayer silica gel column chromatography processing. A

multilayer silica gel column is a column which carried out the laminating of silica gel and the silica gel which covered the potassium hydroxide, the sulfuric acid, the silver nitrate, etc. one by one. Vitriolization / silica gel column chromatography processing may be performed instead of multilayer silica gel column chromatography processing. Moreover, alumina column chromatography processing, activated carbon silica gel column chromatography processing, and high-performance-chromatography (HPLC) processing may be performed as pretreatment if needed.

[0015] By the qualitative analysis (for example, qualitative analysis using a high-resolution mold gas chromatograph (HRGC)) of the dioxin contained in saliva, while being able to clarify existence of the dioxin in saliva, the class of homolog of the dioxin contained in saliva can be identified. Moreover, while being able to clarify quantitative relation of each homolog of the dioxin contained in saliva by the quantitative analysis (for example, quantitative analysis using a high-resolution mold mass spectrometer (HRMS)) of the dioxin contained in saliva, the toxic strength of the dioxin contained in saliva is computable as toxic equivalence (TEQ:Toxicity Equivalency Quantity). Therefore, as the qualitative-analysis result or quantitative-analysis result of the dioxin contained in saliva, the concentration of each homolog of the existence of the dioxin in saliva, the class of homolog of the dioxin contained in saliva, and the dioxin that are contained in saliva, the toxic strength of the dioxin contained in saliva, etc. can be made into an index (toxic equivalence (TEQ)).

[0016] In addition, toxic equivalence (TEQ:Toxicity Equivalency Quantity) is the value which multiplied the amount of each homolog for the toxic strength of the dioxin which exists as mixture of many homologs by each toxic equivalence multiplier (TEF:Toxicity Equivalency Factor). Moreover, a toxic equivalence multiplier is a multiplier which expressed 2, 3 and 7 with the strongest toxicity, and 8-TCDD for the toxic strength of each homolog of dioxin as 1.

[0017] Any analysis of the are recording situation of the dioxin in the inside of the body of an animal, such as detection of the dioxin accumulated in the inside of the body of an animal, qualitative analysis, and quantitative analysis, is included in analysis of the dioxin accumulated in the inside of the body of an animal. For example, the existence of are recording of the dioxin in the inside of the body of an animal is detectable by making existence of the dioxin in saliva into an index. Moreover, the class of homolog of the dioxin accumulated in the inside of the body of an animal can be identified by making into an index the class of homolog of the dioxin contained in saliva. Moreover, quantitative relation of each homolog of the dioxin accumulated in the inside of the body of an animal can be clarified by making into an index quantitative relation of each homolog of the dioxin contained in saliva. Moreover, the dioxin accumulated in the inside of the body of an animal can guess the effect which it can have on the animal concerned by making into an index the toxic strength of the dioxin contained in saliva.

[0018]

[Example] Hereafter, this invention is concretely explained based on an example.

[Example 1] The qualitative analysis and quantitative analysis coplanar [14 kinds of / PCB] which are contained by the following approaches in the saliva extracted from Homo sapiens were carried out.

[0019] 14 kinds of coplanar one PCB made applicable to analysis (1) 4 3, 3', 4'-T four CB (TEF (1994) =0.0005;TEF(1998) =0.0001), (2) 3, 3', 4, 4', 5-P5CB (TEF(1994) =0.1;TEF(1998) =0.1), (3) 3, 3', 4, 4', 5, 5'-H6CB (TEF(1994) =0.01;TEF(1998) =0.01) and (4) 3, 4, 4', 5-T four CB (TEF (1998) =0.0001) (the above four kinds are Non-ortho-PCBs), (5) 2', 3 and 4, 4', 5-P5CB (TEF (1994) =0.0001;TEF(1998) =0.0001), (6) 2, 3', 4, 4', 5-P5CB (TEF(1994) =0.0001;TEF(1998) =0.0001), (7) 2, 3, 3', 4, 4'-P5CB (TEF(1994) =0.0001;TEF(1998) =0.0001), (8) 2, 3, 4, 4', 5-P5CB (TEF(1994) =0.0005;TEF(1998) =0.0005), (9) 2, 3', 4, 4', 5, 5'-H6CB (TEF(1994) =0.00001;TEF (1998) =0.00001), (10) 2, 3, 3', 4, 4', 5-H6CB (TEF(1994) =0.0005;TEF(1998) =0.0005), (11) 2, 3, 3', 4, 4', 5'-H6CB () [TEF] (1994) =0.0005;TEF(1998) =0.0005 and (12) 2, 3, 3', 4, 4', 5, 5'-H7CB (TEF(1994) =0.0001;TEF(1998) =0.0001) (the above eight kinds are Mono-ortho-PCBs), (13) 2, 2', 3 and 4, 4', and 5 and 5 -- they are -H7CB (TEF(1994) =0.00001) and (14) 2, 2', 3, 3', '4, 4', and 5-H7CB (TEF(1994) =0.0001) (the above two kinds are di-ortho-PCBs).

[0020] (1) The instrument used for extraction of the extraction saliva sample of a saliva sample

was washed with the acetone and toluene for a residual-agricultural-chemicals trial, and what was dried completely was used. After brushing cleaned the inside of the oral cavity of healthy people A-E of five young-and-old-of-both-sexes bubble ****, about 100g of saliva was extracted under the condition of not stimulating. In addition, the sex of A is a male, age is 47 years old, the sex of B is a male, age is 37 years old, the sex of C is a woman, age is 36 years old, the sex of D is a woman, age is 35 years old, the sex of E is a woman and age is 32 years old.

[0021] (2) The outline of the pretreatment approach of the pretreatment saliva sample of a saliva sample is shown in drawing 1 .

[0022] 0.2ng addition of 14 kinds of coplanar one PCB which carried out the indicator to the saliva sample by 13C as an internal standard was carried out, respectively.

[0023] It dehydrated and condensed, after extracting the saliva sample which added the internal standard by dichloromethane 100ml (2 times) and rinsing by carrying out centrifugal [of the obtained extract] (2 times). After measuring the fat content of this crude extract, hexane **** was carried out, it applied to the multilayer silica gel column (column which carried out the laminating of silica gel and the silica gel which covered the potassium hydroxide, the sulfuric acid, the silver nitrate, etc. one by one as shown in drawing 1), and the obtained fraction was condensed. This was analyzed by the SIM method using high-resolution mold gas chromatograph-mass spectrometer (HRGC/HRMS), and the analysis result was amended with the recovery of an internal standard.

[0024] (3) Analysis coplanar [PCB] was performed using the gas chromatograph-mass spectrometer (AUTOSPEC ULTIMA GC section: HEWLETT PACKARD HP-6890) made from analysis MICROMASS coplanar [by the SIM method using high-resolution mold gas chromatograph-mass spectrometer (HRGC/HRMS) / PCB], the concentration (pg/g) of each homolog coplanar [PCB] contained in the saliva extracted from A-E was measured, and the toxic equivalence (TEQ) of the dioxin contained in saliva based on this was computed.

[0025] The operating condition of the GC section was carried out as follows. As a separation column, the silica capillary column (60mx0.32mm 0.25micrometer) connected with DB-5MS (J&W) was used. After maintaining column temperature for 1 minute at 150 degrees C, it is raised at 20-degree-C a rate for /to 185 degrees C, subsequently is raised at 2-degree-C a rate for /to 245 degrees C, and after maintaining for 3 minutes at 245 degrees C, it was raised at 6-degree-C a rate for /to 290 degrees C.

[0026] The conditions of the MS section were carried out as follows. the ionization approach -- EI and ionizing voltage -- 295 degrees C and ion source temperature were made into 270 degrees C, and 40V and ionizing current made [500microA and acceleration voltage] resolution 10,000 or more for 8kV and interface temperature. Moreover, the setting mass number was carried out as shown in Table 1.

[0027]

[Table 1]

M+ (M+2)+ (M+4)+ T4CBs 289.9224 291.9195 P5CBs 325.8805 327.8776 H6CBs 359.8415 361.8386 H7CBs 393.8025 395.7996 13C12-T4CBs 301.9626 303.9597 13C12-P5CBs 337.9207 339.917813C12-H6CBs371.8817 373.8788 13C12-T7CBs 405.8428407.8398 [0028] The concentration (pg/g) of each homolog coplanar [PCB] and the toxic equivalence (TEQ) which are contained in the saliva extracted from A-E were as being shown in drawing 2 . In addition, among drawing 2 , "TEF (1994)" expresses the value defined by WHO in 1994, and "TEF (1998)" expresses the value defined by WHO in 1998. Moreover, "Total TEQ1" expresses TEQ obtained using TEF (1994), and "Total TEQ2" expresses TEQ obtained using TEF (1998).

[0029] As shown in drawing 2 , that the determination limit value (value below a limit-of-detection value (0.01 pg/g)) was shown among 14 kinds of coplanar one PCB three -- four -- four -- ' -- five - T four -- CB (3 in 5 samples sample) -- three -- three -- ' -- four -- four -- ' -- five - P -- 5 CB (3 in 5 samples sample) 3, 3', 4, 4', 5, 5'-H6CB (4 in 5 samples sample), two -- ' -- three -- four -- four -- ' -- five - P -- five -- CB (2 in 5 samples sample) -- and -- two -- three -- three -- ' -- four -- four -- ' -- five -- five -- ' - H -- seven -- CB (1 in 5 samples sample) -- it is -- other coplanar one PCB -- qualitative analysis and quantitative

analysis -- having been possible . The qualitative analysis and quantitative analysis of dioxin which are contained in saliva made the specimen the saliva of 10 - 10ml of numbers, and became clear [that it can carry out by the SIM method using high-resolution mold gas chromatograph-mass spectrometer (HRGC/HRMS)] from this result.

[0030] Moreover, since the difference arising from aging (** > **) and sex difference (man > woman) was looked at by the concentration coplanar [PCB] in saliva as shown in drawing 2 , the concentration coplanar [PCB] in saliva became clear [reflecting the are recording situation coplanar / PCB / in the human inside of the body]. That is, by making saliva into a specimen and making into an index the dioxin contained in saliva, including infants - an old man, it was large and it became clear that the are recording situation of the dioxin in the human inside of the body can be analyzed.

[0031] If the concentration (pg/g) of each homolog coplanar [PCB] and the toxic equivalence (TEQ) which are shown in drawing 2 are expressed with a lipid reduced property (concentration (pg/g) and toxic equivalence (TEQ) of each homolog coplanar [PCB] per 1g of lipids which are contained in saliva), it will become as drawing 3 . In addition, since it was A:12mg, B:11mg, C:5mg, D:2mg, and E:5mg when the amount of lipids contained in 100g of each saliva extracted from A-E was measured, the lipid reduced property was calculated based on the amount of lipids in each saliva.

[0032] On the other hand, the concentration (pg/g) of each homolog coplanar [PCB] and the toxic equivalence (TEQ) which are contained in the blood extracted from healthy people F-H are expressed with the lipid reduced property by well-known reference (Chemosphere, Vol.37, Nos 9-12, pp.1773-1780, 1998), and it is quoted by drawing 4 . In addition, "Incl up" means 2' and that 3, 4, 4', and 5-P5CB are contained in the peak of 2, 3', 4, 4', and 5-P5CB among drawing 4 .

[0033] When drawing 3 was compared with drawing 4 , the concentration (pg/g) of each homolog of Plana-PCB and toxic equivalence (TEQ) did not have the difference remarkable at the case where the case where blood is made into a specimen, and saliva are made into a specimen. It became clear from this result that the are recording situation of the dioxin in the human inside of the body can be analyzed in a precision comparable as the case where blood is used as a specimen by using saliva as a specimen.

[0034] It became clear that the dioxin accumulated in the inside of the body of an animal can be analyzed in a precision comparable as the case where blood is made into a specimen by making saliva with a easier sampling than blood into a specimen, and making into an index the dioxin contained in saliva from the above thing. That is, when analyzing the dioxin accumulated in the inside of the body of an animal, it became clear that saliva can be used as a specimen replaced with blood.

[0035]

[Effect of the Invention] According to this invention, the dioxin accumulated in the inside of the body of an animal can be analyzed in a precision comparable as the case where blood is made into a specimen, by making saliva with a easier sampling than blood into a specimen, and making into an index the dioxin contained in saliva.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] However, since about 100ml is needed as blood volume when measuring the concentration of the dioxin in blood, it cannot be targeted at infants. Moreover, since it is that to which blood extraction is performed as part of a medical action, it is difficult to extract many specimens.
[0007] Then, this invention aims at offering the approach of analyzing the dioxin accumulated in the inside of the body of an animal by making into an index the dioxin which contains the specimen which can be analyzed in a precision comparable as the case where a sampling is easy and makes blood a specimen rather than blood as a specimen at the time of analyzing the dioxin accumulated in the inside of the body of an animal in a header and its specimen.

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MEANS

[Means for Solving the Problem] In order to solve the above-mentioned technical problem, analytical method of the dioxin accumulated in the inside of the body of the animal offered by this invention is characterized by analyzing the dioxin accumulated in the inside of the body of said animal by making into an index the dioxin contained in the saliva extracted from the animal.
[0009]
[Embodiment of the Invention] Hereafter, this invention is explained to a detail. In this invention, the dioxin analogue which shows the same toxicity as PCDD or PCDF like coplanar PCB (coplanar PCB) besides Pori chlorination dibenzo-Para-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) is contained in "dioxin."
[0010] PCDD is a compound which has a dibenzo-Para-dioxin frame, and is the compound with which one piece or plurality was permuted by the chlorine atom among the hydrogen atoms of the 1-4th place and the 6-9th place. Moreover, PCDF is a compound which has a dibenzofuran frame and is the compound with which one piece or plurality was permuted by the chlorine atom among the hydrogen atoms of the 1-4th place and the 6-9th place. Moreover, PCB is a compound which has a biphenyl frame, one piece or plurality is the compound permuted by the chlorine atom among the hydrogen atoms the 2-6th place and like 2'-6', and coplanar one PCB is in the condition (coplanarity: Coplanarity) that there are two phenyl groups in the same flat surface in three dimensions in PCB (a non orthochromatic object, a mono-orthochromatic object, and JIORUSO object).
[0011] In this invention, in case the dioxin accumulated in the inside of the body of an animal is analyzed, the saliva extracted from the animal concerned is used as a specimen. The saliva used as a specimen is saliva which the animal used as the candidate for analysis secretes, and is extracted from the animal used as the candidate for analysis. It is not limited especially as long as the animal used as the candidate for analysis can secrete saliva, and as the example, mammalians, such as Homo sapiens, an ape, a dog, a cat, a rat, a guinea pig, and a rabbit, are mentioned.
[0012] The saliva used as a specimen is extractable according to a conventional method from the inside of the oral cavity of the animal used as the candidate for analysis. In case saliva is extracted, it is desirable to clean beforehand the inside of the oral cavity of the animal used as the candidate for analysis by brushing etc. so that the dioxin of the food origin which the animal concerned took in may not mix into the saliva which the animal used as the candidate for analysis secretes. Any of the saliva secreted under the saliva secreted under the conditions of not stimulating, and owner stimulus conditions are sufficient as the saliva to extract. As for the instrument used for extraction of saliva, it is desirable to fully wash by an acetone, toluene, etc. for a residual-agricultural-chemicals trial so that the dioxin adhering to the instrument concerned may not mix into saliva. The output of saliva is a complement at analysis of the dioxin contained in saliva, and is usually 10ml - about 10ml of numbers.
[0013] In this invention, in case you analyze the dioxin accumulated in the inside of the body of an animal, let the dioxin contained in the saliva extracted from the animal concerned be an index. The qualitative-analysis result or quantitative-analysis result of dioxin specifically contained in the saliva extracted from the animal concerned can be made into an index.

[0014] The qualitative analysis and quantitative analysis of dioxin which are contained in saliva can be carried out by the selected-ion-detection method (the SIM method) which used for example, high-resolution mold gas chromatograph-mass spectrometer (HRGC/HRMS). Under the present circumstances, to the saliva sample, the internal standard (for example, several sorts of coplanar PCB which carried out the indicator by 13C or 37Cl(s) when coplanar one PCB was a candidate for analysis) corresponding to the dioxin it is incomparable for the candidate for analysis is added. Moreover, after adding an internal standard to a saliva sample, pretreatment is performed and the analysis interfering substance is removed from the saliva sample. After carrying out solvent extraction (for example, dichloromethane extract) for example, of the saliva sample and condensing an extract, pretreatment is ****(ed) to a hexane and, subsequently can be performed by carrying out multilayer silica gel column chromatography processing. A multilayer silica gel column is a column which carried out the laminating of silica gel and the silica gel which covered the potassium hydroxide, the sulfuric acid, the silver nitrate, etc. one by one. Vitriolization / silica gel column chromatography processing may be performed instead of multilayer silica gel column chromatography processing. Moreover, alumina column chromatography processing, activated carbon silica gel column chromatography processing, and high-performance-chromatography (HPLC) processing may be performed as pretreatment if needed.

[0015] By the qualitative analysis (for example, qualitative analysis using a high-resolution mold gas chromatograph (HRGC)) of the dioxin contained in saliva, while being able to clarify existence of the dioxin in saliva, the class of homolog of the dioxin contained in saliva can be identified. Moreover, while being able to clarify quantitative relation of each homolog of the dioxin contained in saliva by the quantitative analysis (for example, quantitative analysis using a high-resolution mold mass spectrometer (HRMS)) of the dioxin contained in saliva, the toxic strength of the dioxin contained in saliva is computable as toxic equivalence (TEQ:Toxicity Equivalency Quantity). Therefore, as the qualitative-analysis result or quantitative-analysis result of the dioxin contained in saliva, the concentration of each homolog of the existence of the dioxin in saliva, the class of homolog of the dioxin contained in saliva, and the dioxin that are contained in saliva, the toxic strength of the dioxin contained in saliva, etc. can be made into an index (toxic equivalence (TEQ)).

[0016] In addition, toxic equivalence (TEQ:Toxicity Equivalency Quantity) is the value which multiplied the amount of each homolog for the toxic strength of the dioxin which exists as mixture of many homologs by each toxic equivalence multiplier (TEF:Toxicity Equivalency Factor). Moreover, a toxic equivalence multiplier is a multiplier which expressed 2, 3 and 7 with the strongest toxicity, and 8-TCDD for the toxic strength of each homolog of dioxin as 1.

[0017] Any analysis of the are recording situation of the dioxin in the inside of the body of an animal, such as detection of the dioxin accumulated in the inside of the body of an animal, qualitative analysis, and quantitative analysis, is included in analysis of the dioxin accumulated in the inside of the body of an animal. For example, the existence of are recording of the dioxin in the inside of the body of an animal is detectable by making existence of the dioxin in saliva into an index. Moreover, the class of homolog of the dioxin accumulated in the inside of the body of an animal can be identified by making into an index the class of homolog of the dioxin contained in saliva. Moreover, quantitative relation of each homolog of the dioxin accumulated in the inside of the body of an animal can be clarified by making into an index quantitative relation of each homolog of the dioxin contained in saliva. Moreover, the dioxin accumulated in the inside of the body of an animal can guess the effect which it can have on the animal concerned by making into an index the toxic strength of the dioxin contained in saliva.

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EXAMPLE

[Example] Hereafter, this invention is concretely explained based on an example.
[Example 1] The qualitative analysis and quantitative analysis coplanar [14 kinds of / PCB] which are contained by the following approaches in the saliva extracted from Homo sapiens were carried out.
[0019] 14 kinds of coplanar one PCB made applicable to analysis (1) 4 3, 3', 4'-T four CB (TEF (1994) =0.0005;TEF(1998) =0.0001), (2) 3, 3', 4, 4', 5-P5CB (TEF(1994) =0.1;TEF(1998) =0.1), (3) 3, 3', 4, 4', 5, 5'-H6CB (TEF(1994) =0.01;TEF(1998) =0.01) and (4) 3, 4, 4', 5-T four CB (TEF (1998) =0.0001) (the above four kinds are Non-ortho-PCBs), (5) 2', 3 and 4, 4', 5-P5CB (TEF (1994) =0.0001;TEF(1998) =0.0001), (6) 2, 3', 4, 4', 5-P5CB (TEF(1994) =0.0001;TEF(1998) =0.0001), (7) 2, 3, 3', 4, 4'-P5CB (TEF(1994) =0.0001;TEF(1998) =0.0001), (8) 2, 3, 4, 4', 5-P5CB (TEF(1994) =0.0005;TEF(1998) =0.0005), (9) 2, 3', 4, 4', 5, 5'-H6CB (TEF(1994) =0.00001;TEF (1998) =0.00001), (10) 2, 3, 3', 4, 4', 5-H6CB (TEF(1994) =0.0005;TEF(1998) =0.0005), (11) 2, 3, 3', 4, 4', 5'-H6CB () [TEF] (1994) =0.0005;TEF(1998) =0.0005 and (12) 2, 3, 3', 4, 4', 5, 5'-H7CB (TEF(1994) =0.0001;TEF(1998) =0.0001) (the above eight kinds are Mono-ortho-PCBs), (13) 2, 2', 3 and 4, 4', and 5 and 5 -- they are -H7CB (TEF(1994) =0.00001) and (14) 2, 2', 3, 3', '4, 4', and 5-H7CB (TEF(1994) =0.0001) (the above two kinds are di-ortho-PCBs).
[0020] (1) The instrument used for extraction of the extraction saliva sample of a saliva sample was washed with the acetone and toluene for a residual-agricultural-chemicals trial, and what was dried completely was used. After brushing cleaned the inside of the oral cavity of healthy people A-E of five young-and-old-of-both-sexes bubble ****, about 100g of saliva was extracted under the condition of not stimulating. In addition, the sex of A is a male, age is 47 years old, the sex of B is a male, age is 37 years old, the sex of C is a woman, age is 36 years old, the sex of D is a woman, age is 35 years old, the sex of E is a woman and age is 32 years old.
[0021] (2) The outline of the pretreatment approach of the pretreatment saliva sample of a saliva sample is shown in drawing 1 .
[0022] 0.2ng addition of 14 kinds of coplanar one PCB which carried out the indicator to the saliva sample by 13C as an internal standard was carried out, respectively.
[0023] It dehydrated and condensed, after extracting the saliva sample which added the internal standard by dichloromethane 100ml (2 times) and rinsing by carrying out centrifugal [of the obtained extract] (2 times). After measuring the fat content of this crude extract, hexane **** was carried out, it applied to the multilayer silica gel column (column which carried out the laminating of silica gel and the silica gel which covered the potassium hydroxide, the sulfuric acid, the silver nitrate, etc. one by one as shown in drawing 1), and the obtained fraction was condensed. This was analyzed by the SIM method using high-resolution mold gas chromatograph-mass spectrometer (HRGC/HRMS), and the analysis result was amended with the recovery of an internal standard.
[0024] (3) Analysis coplanar [PCB] was performed using the gas chromatograph-mass spectrometer (AUTOSPEC ULTIMA GC section: HEWLETT PACKARD HP-6890) made from analysis MICROMASS coplanar [by the SIM method using high-resolution mold gas chromatograph-mass spectrometer (HRGC/HRMS) / PCB], the concentration (pg/g) of each

homolog coplanar [PCB] contained in the saliva extracted from A-E was measured, and the toxic equivalence (TEQ) of the dioxin contained in saliva based on this was computed.
[0025] The operating condition of the GC section was carried out as follows. As a separation column, the silica capillary column (60mx0.32mm 0.25micrometer) connected with DB-5MS (J&W) was used. After maintaining column temperature for 1 minute at 150 degrees C, it is raised at 20-degree-C a rate for /to 185 degrees C, subsequently is raised at 2-degree-C a rate for /to 245 degrees C, and after maintaining for 3 minutes at 245 degrees C, it was raised at 6-degree-C a rate for /to 290 degrees C.
[0026] The conditions of the MS section were carried out as follows. the ionization approach -- EI and ionizing voltage -- 295 degrees C and ion source temperature were made into 270 degrees C, and 40V and ionizing current made [500microA and acceleration voltage] resolution 10,000 or more for 8kV and interface temperature. Moreover, the setting mass number was carried out as shown in Table 1.
[0027]
[Table 1]
M+ (M+2)+ (M+4)+ T4CBs 289.9224 291.9195 P5CBs 325.8805 327.8776 H6CBs 359.8415 361.8386 H7CBs 393.8025 395.7996 13C12-T4CBs 301.9626 303.9597 13C12-P5CBs 337.9207 339.917813C12-H6CBs371.8817 373.8788 13C12-T7CBs 405.8428407.8398 [0028] The concentration (pg/g) of each homolog coplanar [PCB] and the toxic equivalence (TEQ) which are contained in the saliva extracted from A-E were as being shown in drawing 2 . In addition, among drawing 2 , "TEF (1994)" expresses the value defined by WHO in 1994, and "TEF (1998)" expresses the value defined by WHO in 1998. Moreover, "Total TEQ1" expresses TEQ obtained using TEF (1994), and "Total TEQ2" expresses TEQ obtained using TEF (1998).
[0029] As shown in drawing 2 , that the determination limit value (value below a limit-of-detection value (0.01 pg/g)) was shown among 14 kinds of coplanar one PCB three -- four -- four -- ' -- five - T four -- CB (3 in 5 samples sample) -- three -- three -- ' -- four -- four -- ' -- five - P -- 5 CB (3 in 5 samples sample) 3, 3', 4, 4', 5, 5'-H6CB (4 in 5 samples sample), two -- ' -- three -- four -- four -- ' -- five - P -- five -- CB (2 in 5 samples sample) -- and -- two -- three -- three -- ' -- four -- four -- ' -- five -- five -- ' - H -- seven -- CB (1 in 5 samples sample) -- it is -- other coplanar one PCB -- qualitative analysis and quantitative analysis -- having been possible . The qualitative analysis and quantitative analysis of dioxin which are contained in saliva made the specimen the saliva of 10 - 10ml of numbers, and became clear [that it can carry out by the SIM method using high-resolution mold gas chromatograph-mass spectrometer (HRGC/HRMS)] from this result.
[0030] Moreover, since the difference arising from aging (** > **) and sex difference (man > woman) was looked at by the concentration coplanar [PCB] in saliva as shown in drawing 2 , the concentration coplanar [PCB] in saliva became clear [reflecting the are recording situation coplanar / PCB / in the human inside of the body]. That is, by making saliva into a specimen and making into an index the dioxin contained in saliva, including infants - an old man, it was large and it became clear that the are recording situation of the dioxin in the human inside of the body can be analyzed.
[0031] If the concentration (pg/g) of each homolog coplanar [PCB] and the toxic equivalence (TEQ) which are shown in drawing 2 are expressed with a lipid reduced property (concentration (pg/g) and toxic equivalence (TEQ) of each homolog coplanar [PCB] per 1g of lipids which are contained in saliva), it will become as drawing 3 . In addition, since it was A:12mg, B:11mg, C:5mg, D:2mg, and E:5mg when the amount of lipids contained in 100g of each saliva extracted from A-E was measured, the lipid reduced property was calculated based on the amount of lipids in each saliva.
[0032] On the other hand, the concentration (pg/g) of each homolog coplanar [PCB] and the toxic equivalence (TEQ) which are contained in the blood extracted from healthy people F-H are expressed with the lipid reduced property by well-known reference (Chemosphere, Vol.37, Nos 9-12, pp.1773-1780, 1998), and it is quoted by drawing 4 . In addition, "Incl up" means 2' and that 3, 4, 4', and 5-P5CB are contained in the peak of 2, 3', 4, 4', and 5-P5CB among drawing 4 .
[0033] When drawing 3 was compared with drawing 4 , the concentration (pg/g) of each homolog

of Plana-PCB and toxic equivalence (TEQ) did not have the difference remarkable at the case where the case where blood is made into a specimen, and saliva are made into a specimen. It became clear from this result that the are recording situation of the dioxin in the human inside of the body can be analyzed in a precision comparable as the case where blood is used as a specimen by using saliva as a specimen.

[0034] It became clear that the dioxin accumulated in the inside of the body of an animal can be analyzed in a precision comparable as the case where blood is made into a specimen by making saliva with a easier sampling than blood into a specimen, and making into an index the dioxin contained in saliva from the above thing. That is, when analyzing the dioxin accumulated in the inside of the body of an animal, it became clear that saliva can be used as a specimen replaced with blood.

[Translation done.]

* NOTICES *

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2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing the outline of the pretreatment approach of a saliva sample.
[Drawing 2] It is drawing showing the concentration (pg/g) coplanar [PCB] contained in a saliva sample, and toxic equivalence (TEQ).
[Drawing 3] It is drawing which expressed with the lipid reduced property the concentration (pg/g) coplanar [PCB] contained in a saliva sample, and toxic equivalence (TEQ).
[Drawing 4] It is drawing which expressed with the lipid reduced property the concentration (pg/g) coplanar [PCB] contained in a blood sample, and toxic equivalence (TEQ).

[Translation done.]

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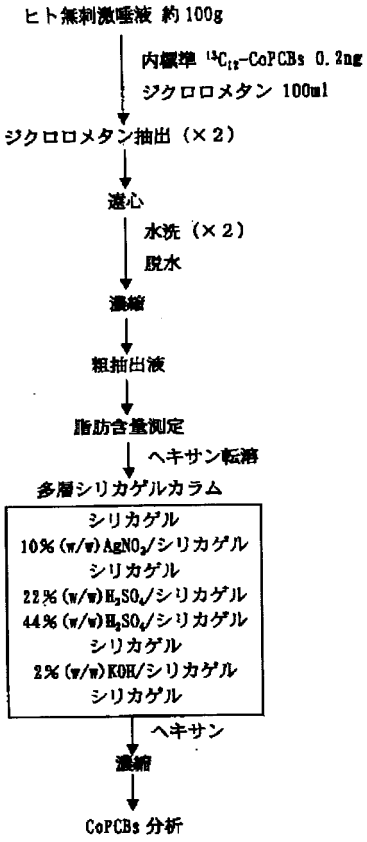
DRAWINGS

[Drawing 4]

	TEF(1994)	F	G	H
3,3',4,4'-T_4CB	0.0005	25	24	18.4
3,3',4,4',5-P_5CB	0.1000	149	134	29.0
3,3',4,4',5,5'-H_6CB	0.0100	84	99	32.2
2,3,3',4,4'-P_5CB	0.0001	11304	8603	3600
2,3,4,4',5-P_5CB	0.0005	2745	2756	2000
2,3',4,4',5-P_5CB	0.0001	43599	41021	17100
2',3,4,4',5-P_5CB	0.0001	676	789	Incl up
2,3,3',4,4',5-H_6CB	0.0005	13962	18216	5400
2,3,3',4,4',5'-H_6CB	0.0005	3131	4092	1700
2,3',4,4',5,5'-H_6CB	0.00001	8325	8455	2700
2,3,3',4,4',5,5'-H_7CB	0.0001	850	925	1300
2,2',3,3',4,4',5-H_7CB	0.0001	17531	20820	15400
2,2',3,4,4',5,5'-H_7CB	0.00001	84082	79759	31200
Total TEQ		33.99	35.03	11.86

[Drawing 1]



[Drawing 2]

	TEF(1994)	TEF(1998)	A	B	C	D	E	blank
3,3',4,4'-T_4CB	0.0005	0.0001	8.6	1.5	0.069	0.057	0.025	N.D.
3,4,4',5-T_4CB	—	0.0001	0.83	0.084	N.D.	N.D.	0.0032	N.D.
3,3',4,4',5-P_5CB	0.1000	0.1000	0.55	0.013	N.D.	0.0049	N.D.	N.D.
3,3',4,4',5,5'-H_6CB	0.0100	0.0100	0.14	N.D.	N.D.	N.D.	0.0079	N.D.
2,3,3',4,4'-P_5CB	0.0001	0.0001	39	1	0.16	0.084	0.11	0.0096
2,3,4,4',5-P_5CB	0.0005	0.0005	3.3	0.22	0.032	0.019	0.022	N.D.
2,3',4,4',5-P_5CB	0.0001	0.0001	77	2.8	0.58	0.28	0.38	0.031
2',3,4,4',5-P_5CB	0.0001	0.0001	7.4	0.096	0.022	0.0059	0.0047	N.D.
2,3,3',4,4',5-H_6CB	0.0005	0.0005	7.1	0.52	0.19	0.12	0.097	N.D.
2,3,3',4,4',5'-H_6CB	0.0005	0.0005	1.8	0.14	0.032	0.022	0.025	N.D.
2,3',4,4',5,5'-H_6CB	0.00001	0.00001	2.9	0.15	0.057	0.026	0.016	N.D.
2,3,3',4,4',5,5'-H_7CB	0.0001	0.0001	0.58	0.055	0.016	0.0092	0.025	N.D.
2,2',3,3',4,4',5-H_7CB	0.0001	—	5.3	0.66	0.18	0.086	0.14	N.D.
2,2',3,4,4',5,5'-H_7CB	0.00001	—	13	2.2	0.55	0.47	0.38	N.D.
Total TEQ[1]			0.080	0.0030	0.00026	0.00040	0.00019	0.0000035
Total TEQ[2]			0.076	0.0023	0.00021	0.00037	0.00017	0.0000035

[Drawing 3]

	TEF (1994)	TEF (1998)	A	B	C	D	E	blank
3, 3', 4, 4'－T_4CB	0.0005	0.0001	72000	14000	1400	2900	500	N.D.
3, 4, 4', 5－T_4CB	－	0.0001	6900	760	N.D.	N.D.	64	N.D.
3, 3', 4, 4', 5－P_5CB	0.1000	0.1000	4600	120	N.D.	250	N.D.	N.D.
3, 3', 4, 4', 5, 5'－H_6CB	0.0100	0.0100	1200	N.D.	N.D.	N.D.	160	N.D.
2, 3, 3', 4, 4'－P_5CB	0.0001	0.0001	330000	9100	3200	4200	2200	0.0096
2, 3, 4, 4', 5－P_5CB	0.0005	0.0005	28000	2000	640	950	440	N.D.
2, 3', 4, 4', 5－P_5CB	0.0001	0.0001	640000	25000	12000	14000	7600	0.031
2', 3, 4, 4', 5－P_5CB	0.0001	0.0001	62000	870	440	300	94	N.D.
2, 3, 3', 4, 4', 5－H_6CB	0.0005	0.0005	59000	4700	3800	6000	1900	N.D.
2, 3, 3', 4, 4', 5'－H_6CB	0.0005	0.0005	15000	1300	640	1100	500	N.D.
2, 3', 4, 4', 5, 5'－H_6CB	0.00001	0.00001	24000	1400	1100	1300	320	N.D.
2, 3, 3', 4, 4', 5, 5'－H_7CB	0.0001	0.0001	4800	500	320	460	500	N.D.
2, 2', 3, 3', 4, 4', 5－H_7CB	0.0001	－	44000	6000	3600	4300	2800	N.D.
2, 2', 3, 4, 4', 5, 5'－H_7CB	0.00001	－	110000	20000	13000	24000	7600	N.D.
Total TEQ[1]			670	27	5.2	20	3.8	0.0000035
Total TEQ[2]			630	21	4.2	19	3.4	0.0000035

[Translation done.]